PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 12565110/VPA/DJH/cmb	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. **PCT**/AU2005/000187	International filing date *(day/month/year)* 14 February 2005	Priority date *(day/month/year)* 12 February 2004

International Patent Classification (IPC) or national classification and IPC

Int. Cl.

C12N 1/20 (2006.01) ***A61K 39/112*** (2006.01) ***A61P 37/04*** (2006.01)

Applicant

THE UNIVERSITY OF QUEENSLAND et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of **4** sheets, as follows:
      - [ ] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:

[X]	Box No. I	Basis of the report
[ ]	Box No. II	Priority
[ ]	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
[ ]	Box No. IV	Lack of unity of invention
[X]	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
[ ]	Box No. VI	Certain documents cited
[ ]	Box No. VII	Certain defects in the international application
[X]	Box No. VIII	Certain observations on the international application

Date of submission of the demand 12 December 2005	Date of completion of this report 01 June 2006
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **JENNIFER FERNANCE** Telephone No. (02) 6283 2269

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/AU2005/000187

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

[X] The international application in the language in which it was filed

[ ] A translation of the international application into , which is the language of a translation furnished for the purposes of:

[ ] international search (under Rules 12.3(a) and 23.1 (b))

[ ] publication of the international application (under Rule 12.4(a))

[ ] international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

[ ] the international application as originally filed/furnished

[X] the description:

pages **1-156** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

[X] the claims:

pages as originally filed/furnished
pages* as amended (together with any statement) under Article 19
pages **157-160** received by this Authority on **13 December 2005** with the letter of **12 December 2005**
pages* received by this Authority on with the letter of

[X] the drawings:

pages **1/32-32/32** as originally filed/furnished
pages* received by this Authority on with the letter of
pages* received by this Authority on with the letter of

[ ] a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages
[ ] the claims, Nos.
[ ] the drawings, sheets/figs
[ ] the sequence listing *(specify)*:
[ ] any table(s) related to the sequence listing *(specify)*:

4. [ ] This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

[ ] the description, pages
[ ] the claims, Nos.
[ ] the drawings, sheets/figs
[ ] the sequence listing *(specify)*:
[ ] any table(s) related to the sequence listing *(specify)*:

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims 6, 8, 9, 17, 21 and 23	**YES**
	Claims 1-5, 7, 10-16, 18-20, 22 and 24-27	**NO**
Inventive step (IS)	Claims 17	**YES**
	Claims 1-16, 18-27	**NO**
Industrial applicability (IA)	Claims 1-27	**YES**
	Claims -	**NO**

2. Citations and explanations (Rule 70.7)

The following documents identified in the International Search Report have been considered for the purposes of this report:

D2: US 6136325 D3: Bjorkman et al

New Citation

D5: Linde K et al (1998) Vet Micro Vol 62 pages 121-134 "Bacterial live vaccines with graded level of attenuation achieved by antibiotic resistance mutations: transduction experiments on the functional unit of resistance, attenuation and further accompanying markers".

Novelty Claims 1-5, 7, 10-16, 18-20, 22 and 24-27

Claims 1-23, 25 and 26 are directed the bacteria *per se*. The limitation that the agent has specific invasive or protective activities does not limit the use of the bacteria in methods using those activities. Therefore a citation that discloses bacteria having the defined mutations as presently described and/or defined would inherently have the defined attributes.

D3 discloses spontaneous rifampicin (Rif), streptomycin (Stm) or nalidixic acid (NaI) resistant S typhimurium mutants (page 123) that may or may not have other identifiable characteristics. It discloses that spontaneous mutants resistant to Rif or NaI in *Salmonella spp* has been mapped to the rpoB gene and gyrA genes, respectively. The use of these strains in vaccines is clearly envisaged (Abstract, Introduction and Discussion).

D5 discloses live attenuated bacteria produced from metabolic drift mutants prepared from both wild type bacteria (donor) and transduced bacteria (pages 123). They comprise those having Rif or NaI resistance and were found to be attenuated (Tables 2 and 5). They are extracted from faecal samples (page 126) and have a mutation in the Rif or NaI genes. The disclosed bacteria are those presently described and defined and therefore are considered to be able to infect stock animals and to colonise and invade the organs as presently defined.

D2 discloses attenuated *Salmonella spp* having Rif and NaI resistance and their use in vaccinating livestock, chickens and cattle (col 7, 9). The markers discussed by the Applicant are the resistance genes themselves. The attenuated bacteria are prepared by the selection of metabolic drift mutants resistant to Rif and/or NaI prepared from both mutated and wild type bacteria (see Examples and Figures). The mutants may or may not have another marker. However, the present claims do not exclude other metabolic drift markers.

(Continued in Supplemental Box)

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The claims are not supported by the description. There is no support for:

- The disclosed bacteria having a reduced capacity to grow and replicate in the presence of bile acids;
- any attenuated bacteria having the required growth characteristics as having the required immunological activity;
- any attenuated bacteria having Rif or NaI resistance having the required growth characteristics and immunological activity;
- any double mutation having the required growth characteristics as providing the required immunological activity (the Examples disclose that specific double mutations are lethal in 50% of cases (RNM29) or are toxic when administered (RNM 4));
- the use of the attenuated bacteria as a carrier for an introduced antigen;
- the vaccination of a human.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box V

Each of the citations and the present specification discloses the use of growth media without the addition of bile acids to determine the growth rate of the bacteria. Each discloses a retarded growth pattern compared to the wild-type. Therefore it is considered that the disclosed bacteria would have a reduced capacity to grow and replicate in the presence of bile acids. Due to the species, natural modification and the attenuated bacteria disclosed in the citations, it is considered that they penetrate and colonise the defined organs as presently defined. The Skilled Addressee would appreciate that the form of the culture is immaterial to the working of the invention. The identification of the type of response elicited by a formulation does not confirm novelty on known formulation or known methods. Therefore claims 1-5, 7, 10-16, 18-20, 22 and 24-27 lack novelty in light of D2, D3 and D5.

Claims 6, 8, 9, 17, 21, 23 meet the criteria set forth in PCT Article 33(2) for novelty. The prior art published before the priority date does not disclose Salmonella dublin or the use of the define agent as a carrier for an introduced antigen. Therefore the subject matter of these claims is new and meets the requirements of Article 33(2) PCT with regard to novelty.

Inventive Step Claims 1-27

Claims 1-5, 7, 10-14, 16, 18-20, 22, 24-27 as for novelty.

In absence to the contrary, the teachings of D2, D3 or D5 is applicable to other strains of Salmonella including S. dublin. Therefore claims 6, 8, 9, 14, 22, 21and 23 lack inventive step.

Industrial Applicability (IA) Claims 1-27

The invention defined in the claims is considered to meet the requirements of Industrial Applicability under Article 33(4) of the PCT because it can be made by, or used in, industry 6, 8, 9, 17, 21 and 23.

CLAIMS

1. A therapeutic agent comprising a live attenuated microorganism produced by selecting from metabolic drift mutants of a wild strain of a microorganism an attenuated microorganism that has a reduced capacity to grow and replicate in the presence of bile salts as compared to the wild strain, wherein the attenuated microorganism is capable of inducing an immune response in the subject to the wild strain.

2. The therapeutic agent of Claim 1 wherein the attenuated microorganism penetrates an organ selected from liver, spleen and gall bladder after parenteral administration of the therapeutic agent.

3. The therapeutic agent of Claim 1 wherein the attenuated microorganism colonises an organ selected from liver, spleen and gall bladder in lower numbers than the wild strain after parenteral administration.

4. The therapeutic agent of Claim 1 wherein the attenuated microorganism is a member of the Enterobacteriaceae.

5. The therapeutic agent of Claim 4 wherein the attenuated microorganism is a *Salmonella sp.*

6. The therapeutic agent of Claim 1 wherein the attenuated microorganism is *Salmonella dublin.*

7. The therapeutic agent of Claim 2 wherein the attenuated microorganism comprises a sequence alteration in an *rpoB* gene.

8. The therapeutic agent of Claim 7 wherein the attenuated microorganism is selected from N-RM4, N-RM8, N-RM9, N.RM15, N-RM20, N-RM25, N-RM27 and R-NM29.

9. The therapeutic agent of Claim 7 wherein the attenuated microorganism is N-RM25.

10. The therapeutic agent of Claim 1 wherein the subject is a livestock animal.

11. The therapeutic agent of Claim 10 wherein the livestock animal is selected from the list consisting of a cow, a sheep and a pig.

12. The therapeutic agent of Claim 1 wherein the subject is a laboratory test animal.

13. The therapeutic agent of Claim 12 wherein the laboratory test animal is selected from the list consisting of a mouse, a rat, a rabbit and a guinea pig.

14. The therapeutic agent of Claim 1 wherein the subject is a human.

15. The therapeutic agent of Claim 1 wherein the metabolic drift mutants are produced by exposing the wild strain to nalidixic acid and rifampicin or chemical or functional equivalents thereof for a time and under conditions sufficient to induce a metabolic-drift mutation.

16. The therapeutic agent of Claim 1 wherein the immune response is directed to an antigen that is naturally occurring with the microorganism.

17. The therapeutic agent of Claim 1 wherein the immune response is directed to an antigen that is introduced to the microorganism.

18. The therapeutic agent of Claim 1 wherein the attenuated microorganism induces a humoral and/or T-cell-mediated immune response.

19. The therapeutic agent of Claim 1 wherein the attenuated microorganism induces a mucosal immune response.

20. The therapeutic agent of Claim 1 wherein the attenuated microorganism is capable of inducing an immune response in the subject to another species of microorganism.

21. The therapeutic agent of Claim 20 wherein the attenuated microorganism is *Salmonella dublin* is capable of inducing an immune response in the subject to *Salmonella typhimurium*.

22. A therapeutic agent comprising a live attenuated *Salmonella* species produced by selecting from metabolic drift mutants of a wild strain of a *Salmonella* species an attenuated *Salmonella* species that has a reduced capacity to grow and replicate in the presence of bile salts as compared to the wild strain, wherein the attenuated *Salmonella* species is capable of inducing an immune response to itself or to an antigen produced thereby or to the wild strain.

23. The therapeutic agent of Claim 22 wherein the *Salmonella sp.* is *Salmonella dublin*.

24. A method of vaccinating a subject against a microorganism or an antigen produced by a microorganism, the method comprising exposing a wild strain of the microorganism to nalidixic acid and rifampicin or their chemical or functional equivalents for a time and under conditions sufficient to produce metabolic drift mutants of the wild strain, which are resistant to nalidixic acid and rifampicin, and selecting from the metabolic drift mutants an attenuated microorganism that has a reduced capacity to grow and replicate in the presence of bile salts as compared to the wild strain, and administering the attenuated microorganism to the subject under conditions sufficient for the attenuated microorganism to migrate to an environment comprising the bile salts where it maintains itself for a time sufficient to induce an immune response to the microorganism or an antigen produced thereby.

25. A purified culture of a *Salmonella* species as defined in claim 22.

26. The purified culture of Claim 25 wherein the culture is freeze dried, frozen or reconstituted.

27. Use of the purified culture of Claim 25 or 26 in the manufacture of a vaccine to induce an immune response in a mammal to a *Salmonella* species.